PRINCETON SEPARATIONS

CENTRI • SPIN™-20 COLUMNS

For Research Use Only

PRINCIPLE

CENTRI • SPIN™-20 Columns are used for the fast and efficient purification of large molecules (peptides, proteins, nucleic acids, complex carbohydrates) from small molecules (nucleotides, labels and buffer salts). The column design is based on the description by Sambrook, *et al.* (1) of gel filtration for the purification of DNA from nick translation reactions. Each unit consists of a special fritted microfuge tube, dry gel, wash tube and sample collection tube.

The gel will provide excellent recovery (>70%) of DNA fragments **>20-mer or 20 base pairs** while removing >98% of salts, NTP's and other low-molecular-weight compounds.

The column gel is **hydrated** with reagent-grade water or a suitable buffer and spun in a microcentrifuge or swinging-bucket centrifuge to **remove the interstitial fluid**. The sample is then applied and the column is spun again, **processing the sample.** The sample is purified by the retention of low-molecular-weight contaminants in the matrix, while the larger molecules of interest are exchanged into the buffer of choice and eluted into the collection tube.

These columns are far superior — in **ease of use, speed, and non-toxicity** — to such common techniques as phenol/chloroform extraction, ethanol precipitation, dialysis and ultrafiltration.

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Benefits include:

- **RAPID AND EFFICIENT SEPARATIONS**
- **BUFFER NOT PRESELECTED**
- **COLUMNS STABLE AT ROOM TEMPERATURE**
- **CONVENIENT 20-50µl SAMPLE SIZE**

CENTRIFUGE NOTES

Maximum yield and efficiency are obtained with the horizontal or swinging-bucket rotors. However, fixed-angle-rotor microcentrifuges provide acceptable performance and save time.

On a variable speed microcentrifuge, DO NOT use the pulse button, which overrides the speed setting and takes the rotor to maximum g-force. If you are not sure of the g-force generated by your centrifuge at specific speeds, calculate the correct speed by using the following formula:

$$\text{rpm} = \sqrt{\frac{\text{RCF}}{(1.119 \times 10^{-5})\, r\ (\text{cm})}}$$

Where

rpm = revolutions per minute;
RCF = Relative Centrifugal Force

and

r = radius (cm) measured from center of spindle to bottom of rotor bucket.

Example:

For RCF = 750 and r = 7.5 cm

$$\text{rpm} = \sqrt{\frac{750}{(1.119 \times 10^{-5})\,(7.5)}} = 2990 \text{ rpm}$$

QUALITY CONTROL: Every batch of CENTRI • SPIN-20 Columns is tested for separation efficiency and fill accuracy.

MATERIALS PROVIDED

- CENTRI • SPIN-20 Columns containing dry gel
- Wash Tubes (2 ml)
- Sample Collection Tubes (1.5 ml)

ADDITIONAL MATERIALS RECOMMENDED

- Microcentrifuge (Eppendorf 5415C, Variable Speed or equivalent)
- Variable pipets (Pipetman 100 µl)
- Pipet Tips
- Pipet Bulb, Dispo, 2ml Latex
- Microtube Rack
- Vortex Mixer

COMMON PROBLEMS

(1) A failure to remove excess interstitial fluid after hydration of the columns.

(2) Touching the side of the column during sample application.

Both errors can result in ineffective separation.

SOLUTIONS

(1) Note if any columns have released less fluid than the others during the first spin. Simply spinning them again briefly will usually remove the excess fluid.

(2) Load the sample directly into the center of the gel bed and do not touch the sample to the walls of the column.

REFERENCE

Sambrook, J., Fritsch, E.F., and Maniatis, T., *Molecular Cloning : A Laboratory Manual,* Cold Spring Harbor Laboratory, 1989.

CATALOG NO.	SIZE
CS-200	20 pack
CS-201	50 pack

CENTRI • SPIN-20 Protocol

CENTRI • SPIN-20 Columns have been designed specifically for the following uses:

- Removal of free and labeled dNTP's from DNA/RNA as in:
  - nick translation
  - end-labeling reactions
  - PCR reactions
- Primer removal
- Removal of hexamers and octamers from primer-walking and random primer labeling.
- Desalting, removal of traces of phenol or exchange of buffer salts, as in multiple restriction digestions
- Purification/desalting of proteins

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The following protocol may be used for all recommended applications.

1.0 Column Hydration

1.1 Gently tap the column to insure that the dry gel has settled in the bottom of the spin column.

1.2 Remove the top column cap and reconstitute the column by adding **0.65 ml** of reagent grade water or buffer of choice. Replace the column cap and vortex vigorously for ~ 5 seconds. Remove air bubbles by sharply tapping the bottom of the column. It is important to hydrate all of the dry gel.

1.3 Allow at least 30 minutes of room temperature hydration time before using the columns. Reconstituted columns may be stored refrigerated at 4°C for several days. Longer storage can be accomplished in 10 mM sodium azide (NaN_3). **Allow refrigerated columns to warm to room temperature before use.**

2.0 Removal of Interstitial Fluid

2.1 After the gel has hydrated and is free of bubbles, first remove the top column cap, and then remove the column end stopper from the bottom.

2.2 Spin the column and wash tube in a variable speed centrifuge at **750 g for 2 minutes** to remove interstitial fluid. (For example, for Eppendorf Model 5415C, spin at 3000 rpm for 2 minutes.) **If you use a fixed-angle microcentrifuge, keep track of the position of the column using the orientation mark molded into the column.**

2.3 If there is a drop at the end of the column, blot it dry. Discard the wash tube and the interstitial fluid. Do not allow the gel material to dry excessively. **Process the sample within the next few minutes.**

Experimental Results

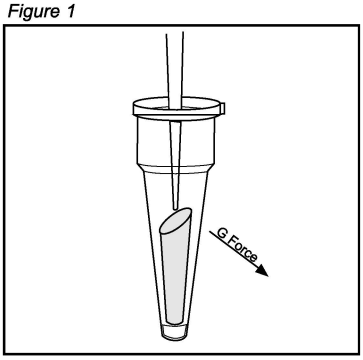
Size of DNA	% Recovery
NTP	0
11-mer	<2
15-mer	39
20-mer	72
24-mer	71
28-mer	81

3.0 Sample Processing

3.1 Hold the column up to the light. Transfer 20 to 50 µl of the sample to the top of the gel. Carefully dispense the sample DIRECTLY ONTO THE CENTER OF THE GEL BED at the top of the column, without disturbing the gel surface (See Figure 1). DO NOT contact the sides of the column with the reaction mixture or the sample pipet tip, since this can reduce the efficiency of purification.

3.2 Place the column into the SAMPLE COLLECTION TUBE (1.5 ml) and place both into the rotor. **Maintain proper column orientation.** The highest point of the gel media in the column should always point toward the outside of the rotor. (See Figure 1.) Spin the column and collection tube at **750 g for 2 minutes**. The purified sample will collect in the bottom of the Sample Collection Tube. Discard the spin column and continue with your procedure.

Figure 1



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